Solvolysis of Model Compounds for α-Hydroxylation of N′-Nitrosonornicotine and 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone: Evidence for a Cyclic Oxonium Ion Intermediate in the Alkylation of Nucleophiles[1]

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N′-Nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), two potent tobacco-specific carcinogens, have been previously found to pyridyloxobutylate DNA. The adducts were found to be unstable and have not been fully characterized. In order to gain an understanding of the chemistry of the pyridyloxobutylating species, five model pyridyloxobutylating agents have been solvolyzed and the products identified. 4-[(Acetoxymethyl)nitrosamino]-1-(3-pyridyl)-1-butanone (3), 4-(carbethoxynitrosamino)-1-(3-pyridyl)-1-butanone (4), 4-oxo-4-(3-pyridyl)-1-butyl *p*-toluenesulfonate (16), 2-chloro-2-(3-pyridyl)-2,3,4,5-tetrahydrofuran (17), and 4-[(acetoxymethyl)nitrosamino]-1-(3-pyridyl)-1-butanol (20) were solvolyzed in buffer and in buffer containing 20% MeOH. The solvolyses of 16 and 17 in H_2O produced only 4-hydroxy-1-(3-pyridyl)-1-butanone (7). In the presence of 20% MeOH, 7 and 2-methoxy-2-(3-pyridyl)-2,3,4,5-tetrahydrofuran (12) were produced from 16 and 17 in a 4:1 ratio. The solvolysis of 3 and 4 in the presence of esterase gave similar products. 4-Methoxy-1-(3-pyridyl)-1-butanone (8) was not detected as a product. In the absence of MeOH, compound 7, 3-pyridyl cyclopropyl ketone (10), and 1-(3-pyridyl)-but-2-en-1-one (18) were observed. In the presence of MeOH, 12 was also formed and the ratio of 7 to 12 was again about 4:1. The esterase-catalyzed hydrolysis of 20 yielded 1-(3-pyridyl)-1,4-butanediol (22), 1-(3-pyridyl)-1,3-butanediol (27), 1-(3-pyridyl)-but-3-en-1-ol (25), 1-(3-pyridyl)but-2-en-1-ol (26), and 2-(3-pyridyl)-2,3,4,5-tetrahydrofuran (24). 4-Methoxy-1-(3-pyridyl)-1-butanol (23) was observed in low yields when the solvolysis of 20 was performed in the presence of MeOH. Hydrolyses of compounds 3 and 20 in deuterated buffer showed that deuterium incorporation was proportional to the buffer concentration. The results indicate that the 4-oxo-4-(3-pyridyl)-1-butanediazonium ion (2), which is formed by metabolism of NNN and NNK, can cyclize to form an electrophilic cyclic oxonium ion (13) which can react with cellular nucleophiles.

Introduction

The tobacco-specific nitrosamines, *N*′-nitrosonornicotine (NNN)[2] and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), are among the most potent carcinogens in cigarettes and other tobacco products (*1*, *2*). NNK and NNN are not direct-acting carcinogens but are believed to be activated by cytochrome P-450 mixed-function oxidases which hydroxylate the carbon atoms adjacent to the nitrosamine group (*1*, *2*). The α-hydroxynitrosamine is unstable and decomposes to a reactive diazohydroxide and an aldehyde. As illustrated in Scheme I, hydroxylation of the methyl group of NNK would produce a pyridyloxobutylating agent, while hydroxylation of the methylene group would result in the formation of a methylating agent. Hydroxylation of the 2′- and 5′-carbons of NNN would produce two different pyridyloxobutylating species. Consistent with this proposal are the observations that NNK both methylates (*3*, *4*) and pyridyloxobutylates DNA (*5*) while NNN pyridyloxobutylates DNA (*5*).

The major methyl DNA adducts derived from NNK were readily characterized as 7- and O^6-methylguanine (*3*, *4*). However, the identification of pyridyloxobutyl adducts has been difficult. Earlier studies demonstrated that up to half of the radioactivity bound to DNA isolated from rats treated with pyridyl-tritiated NNK or NNN was released as 4-hydroxy-1-(3-pyridyl)-1-butanone (7, Scheme II) upon neutral thermal, acid, or enzyme hydrolysis of the DNA (*5*, *6*). These results suggest that the pyridyloxobutyl group was bound to the DNA through an unstable linkage.

In order to better understand the chemistry of the proposed reactive pyridyloxobutylating intermediate, the solvolysis reactions of a number of model compounds were investigated. The inherent instability of the α-hydroxylated metabolites precludes their direct use. Therefore, model compounds were chosen for their ability to generate in situ one or more of the proposed intermediates formed in the decomposition pathways of the α-hydroxylated metabolites of NNK and NNN. These studies concentrated on the pathways resulting from the hydroxylation of the methyl group of NNK and the 2′-carbon of NNN. The products resulting from the 5′-hydroxylation of NNN

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[2] Abbreviations: NNN, *N*′-nitrosonornicotine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol.

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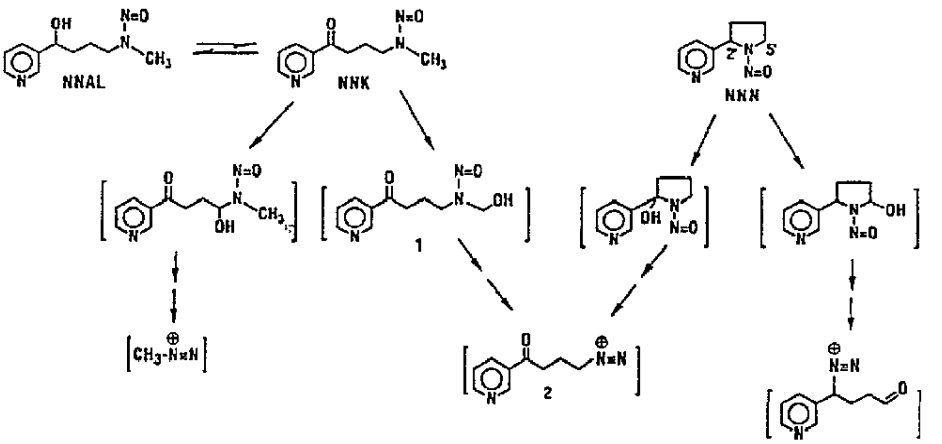
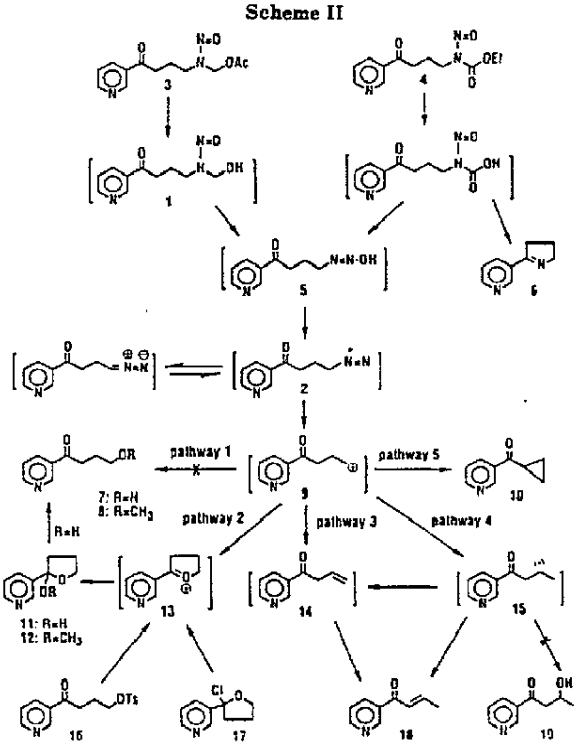
Scheme I



were not investigated in these studies since there is presently no evidence to support the importance of this pathway with respect to DNA alkylation. The compounds chosen for the investigation of the pyridyloxobutylating agent were 4-[(acetoxymethyl)nitrosamino]-1-(3-pyridyl)-1-butanone (3), 4-(carbethoxynitrosamino)-1-(3-pyridyl)-1-butanone (4), 4-oxo-4-(3-pyridyl)-1-butyl *p*-toluenesulfonate (16), and 2-chloro-2-(3-pyridyl)-2,3,4,5-tetrahydrofuran (17). We also studied the solvolysis of 4-[(acetoxymethyl)nitrosamino]-1-(3-pyridyl)-1-butanol (20), a model compound for the methyl hydroxylation of the tumorigen 4-[(acetoxymethyl)nitrosamino]-1-(3-pyridyl)-1-butanol (NNAL) (*7*, *8*), a major metabolite of NNK (*1*, *2*).

A possible intermediate in the hydrolysis of 3, 4, 16, or 17 is the cyclic oxonium ion 13. The reaction of 13 with H_2O would yield the unstable hemiacetal 11 which would rearrange to the keto alcohol 7. However, if 13 were trapped by MeOH, the resulting cyclic ketal 12 would be stable and would be readily detected. Therefore, the various model pyridyloxobutylating agents were also solvolyzed in the presence of 20% MeOH.

Scheme II



Experimental Section

General Procedures. ^1H and ^{13}C NMR spectra were recorded on a Bruker AM360WB NMR spectrometer using tetramethylsilane as an internal standard. Mass spectra were recorded on a Hewlett-Packard Model 5988 spectrometer. UV spectra were recorded on a Hewlett-Packard Model 8452A diode array spectrometer. The HPLC apparatus has been previously described (*9*). The porcine liver esterase (type I) was purchased from Sigma.

Syntheses. 4-(Carbethoxynitrosamino)-1-(3-pyridyl)-1-butanone (4) (*10*), 4-oxo-4-(3-pyridyl)-1-butyl *p*-toluenesulfonate (16) (*11*), 1-(3-pyridyl)-but-2-en-1-one (18) (*12*), 2-methoxy-2-(3-pyridyl)-2,3,4,5-tetrahydrofuran (12) (*11*), 3-hydroxy-1-(3-pyridyl)-1-butanone (19) (*13*), 1-(3-pyridyl)-1,4-butanediol (22) (*14*), and myosmine (6) (*15*) were synthesized by previously described procedures. The purity of the synthetic compounds was judged to be greater than 98% by examination of the ^1H NMR spectra and the HPLC chromatograms.

(A) 4-Hydroxy-1-(3-pyridyl)-1-butanone (7). Compound 7 was synthesized by a modification of a previously described procedure (*16*). γ-Butyrolactone (8.4 mL, 0.11 mol) and ethyl nicotinate (15 mL, 0.11 mol) were added to a stirred suspension of NaH (4.4 g, 60% in mineral oil, 0.11 mol) in 100 mL of anhydrous DMSO. After being stirred overnight at room temperature under a nitrogen atmosphere, the solution was poured into 200 mL of 10% NH_4Cl and extracted into methylene chloride (5 × 100 mL). The solvent was evaporated, 150 mL of concentrated HCl was added, and the reaction mixture was stirred overnight. The acidic solution was neutralized with 10 N NaOH and then extracted with methylene chloride (15 × 100 mL). The combined organic layers were dried over $MgSO_4$, filtered, and evaporated. The oil was dissolved in a minimum of methylene chloride, and it was applied to a 5 × 15 cm flash silica gel column. The column was eluted with 1 L of methylene chloride, then 0.5 L of methylene chloride/ethyl acetate (4/1), 0.5 L of methylene chloride/ethyl acetate (1/1), and 2 L of ethyl acetate. The product was eluted with ethyl acetate/MeOH (19/1) in 20% yield (3.2 g) as a pale yellow oil.

(B) Cyclopropyl 3-Pyridyl Ketone (10) (*17*). K_2CO_3 (14.8 mg, 107 mmol) was added to 34.5 mg of 16 (108 mmol) dissolved in 5 mL of MeOH, and the suspension was stirred for 2 h at room temperature. The solvent was evaporated and the product was purified by flash chromatography with elution by chloroform, in 75% yield (12 mg, 82 mmol): ^1H NMR (360 MHz, $CDCl_3$) δ 9.10 (1 H, d, 2-pyridyl), 8.60 (1 H, d/d, 6-pyridyl), 8.07 (1 H, d/t, 4-pyridyl), 7.26 (1 H, d/d, 5-pyridyl), 2.53 (1 H, m, methine), 1.12 (2 H, m, methylene), 0.89 (2 H, m, methylene); ^{13}C NMR (360 MHz, $CDCl_3$) δ 199.0 (C=O), 153.1 (2-pyridyl), 148.9 (6-pyridyl), 135.2 (4-pyridyl), 132.2 (3-pyridyl), 123.7 (5-pyridyl), 17.0

(methine), 11.6 (methylene); MS *m/e* (relative intensity) 147 (M^+, 24), 118 (6), 106 (M^+ – cyclopropyl, 100), 84 (40), 78 (84), 66 (70), 51 (70), 41 (48).

(C) 2-Chloro-2-(3-pyridyl)-2,3,4,5-tetrahydrofuran (17). Compound 7 (100 mg, 0.6 mmol) was dissolved in 10 mL of anhydrous THF, and the mixture was saturated with anhydrous HCl. It was heated under reflux for 2 h under a nitrogen atmosphere. The solution was evaporated, and 20 mL of saturated $NaHCO_3$ was added. The mixture was then extracted with methylene chloride. The organic layer was dried with $MgSO_4$, filtered, and evaporated to yield 17 in 80% yield: ^1H NMR (360 MHz, $CDCl_3$) δ 8.60 (1 H, d, 2-pyridyl), 8.45 (1 H, d/d, 6-pyridyl), 7.72 (1 H, d/t, 4-pyridyl), 7.26 (1 H, d/d, 5-pyridyl), 4.05 (2 H, m, OCH_2CH_2), 2.30 (1 H, m), 2.17 (1 H, m), 1.97 (1 H, m), 1.74 (1 H, m).

(D) 3-Methoxypropyl Methanesulfonate. 3-Methoxy-1-propanol (1.07 g, 11.9 mmol) was dissolved in 10 mL of anhydrous pyridine and cooled to 0 °C. Methanesulfonyl chloride (1.2 mL, 16 mmol) and then triethylamine (1.0 mL, 13 mmol) were added. The reaction mixture was stirred at 0 °C for 3 h and then poured into 50 mL of 1 M $NaHCO_3$. This mixture was stirred for 5 min and then extracted with chloroform. The combined organic layers were dried with $MgSO_4$, filtered, and evaporated to yield 1.6 g of product in 78% yield: ^1H NMR (360 MHz, $CDCl_3$) δ 4.21 (2 H, t, J = 6.29 Hz, $CH_2CH_2OSO_2CH_3$), 3.38 (2 H, t, J = 5.90 Hz, $CH_2CH_2OCH_3$), 3.32 (3 H, s, OCH_3), 2.91 (3 H, s, SO_2CH_3), 1.89 (2 H, m, $CH_2CH_2CH_2$).

(E) 4-Methoxy-1-(3-pyridyl)-1-butanone (8). 3-Methoxypropyl methanesulfonate (1.3 g, 7.7 mmol) and 2.4 g of NaI (16 mmol) were dissolved in 5 mL of acetone which had been dried over silica gel. The solution was stirred under a nitrogen atmosphere for 16 h at 22 °C. Sodium methanesulfonate precipitated during the course of the reaction. The suspension was poured into H_2O and extracted with chloroform, which was then dried over $MgSO_4$, filtered, and evaporated to give 0.99 g of 1-iodo-3-methoxypropane as a liquid in 63% yield. It was used without further purification.

n-Butyllithium (1.0 mL of 2.5 M in hexane) was added to 10 mL of anhydrous THF under a nitrogen atmosphere at −78 °C. Diisopropylamine (0.35 mL, 2.5 mmol) was then added. After 5 min of stirring, 0.52 g of 2-(3-pyridyl)-2-[(trimethylsilyl)oxy]-acetonitrile (2.5 mmol) (*12*) was added over 5 min. The color of the solution became deep red as the anion was formed. After 15 min, crude 1-iodo-3-methoxypropane (0.50 g, 2.5 mmol) was added. The reaction mixture was stirred for 2.5 h, then poured into 20 mL of 1 M $NaHCO_3$, and extracted with chloroform. The chloroform was evaporated, and the oil was dissolved in 2 mL of 1.0 M HCl and stirred at room temperature for 2 h in order to remove the ketone protecting group. The solution was neutralized with NaOH and extracted with chloroform. The chloroform was dried with $MgSO_4$, filtered, and evaporated. The product was purified by flash chromatography. Impurities were eluted from the column with methylene chloride and the product with ethyl acetate/methylene chloride (1/1) in 10% yield: ^1H NMR (360 MHz, $CDCl_3$) δ 9.18 (1 H, d, J = 1.54 Hz, 2-pyridyl), 8.77 (1 H, d/d, J = 4.83, 1.70 Hz, 6-pyridyl), 8.24 (1 H, d/t, J = 7.97, 2.00 Hz, 4-pyridyl), 7.41 (1 H, d/d, J = 7.90, 4.83 Hz, 5-pyridyl), 3.47 (2 H, t, J = 5.98 Hz, $CH_2CH_2OCH_3$), 3.33 (3 H, s, OCH_3), 3.09 (2 H, t, J = 7.14, $COCH_2CH_2$), 2.04 (2 H, m, $CH_2CH_2CH_2$).

(F) 4-[(Acetoxymethyl)nitrosamino]-1-(3-pyridyl)-1-butanone (3) (*18*). Myosmine (2.00 g, 13.7 mmol) was dissolved in 100 mL of 1 N HCl and left stirring at room temperature for 2 h in order to form the ring-opened compound (*19*). The mixture was concentrated in vacuo. Absolute ethanol was then added and evaporated three times to remove H_2O. Paraformaldehyde (407 mg, 13.7 mmol) was dissolved in 15 mL of warm glacial acetic acid. The resulting solution was cooled to room temperature and added to the ring-opened myosmine which had been suspended in 7 mL of glacial acetic acid. The solution was heated to 35 °C, stirred at this temperature for 1 h, and then stirred overnight at room temperature. A precipitate formed, which was redissolved by warming the mixture. After 1.5 h at 35 °C, the reaction mixture was cooled to 15 °C in an ice bath, and $NaNO_2$ (122 mg, 17.7 mmol), dissolved in a minimum amount of H_2O, was added dropwise over 10 min. The reaction was stirred in the ice bath for 30 min and then stirred at room temperature for another 30 min. The pH of the solution was adjusted to 7 with saturated $NaHCO_3$, and the solution was extracted four times with 100 mL of chloroform. The combined organic layers were washed with saturated $NaHCO_3$, dried over anhydrous K_2CO_3, filtered, and concentrated to give 1.74 g of an oil. The oil was immediately applied to a Florisil column (120 g), eluted with 500 mL of methylene chloride and 500 mL of methylene chloride/ethyl acetate (9/1), and the product (0.34 g), which was approximately 75% pure, was eluted with methylene chloride/ethyl acetate (8/2). This step was performed immediately after extraction as it removes an agent which decomposed compound 3. The product was then purified by preparative TLC on silica gel with development by ethyl acetate/chloroform (1/1). The product, which had an R_f of 0.2, was eluted off the silica gel with acetone and obtained as a pale yellow oil in 20% yield (0.158 g): ^1H NMR ($CDCl_3$) δ 9.16 (1 H, d, 2-pyridyl), 8.80 (1 H, d/d, 6-pyridyl), 8.21 (1 H, d/t, 4-pyridyl), 7.45 (1 H, d/d, 5-pyridyl), 6.20 [1.8 H, s, (*E*)-CH_2OAc], 5.40 [0.2 H, s, (*Z*)-CH_2OAc], 4.40 [0.2 H, t, (*Z*)-CH_2CH_2NNO], 3.72 [1.8 H, t, (*E*)-CH_2CH_2NNO], 3.11 [0.2 H, t, (*Z*)-CH_2CO], 2.96 [1.8 H, t, (*E*)-CH_2CO], 2.30 [0.2 H, m, (*Z*)-$CH_2CH_2CH_2$], 2.14 [2.7 H, s, (*E*)-CH_3CO], 2.10 [0.3 H, s, (*Z*)-CH_3CO], 1.95 [1.8 H, m, (*E*)-$CH_2CH_2CH_2$]; MS EI *m/e* (relative intensity) 206 (M^+ – acetoxy, 16), 193 (8), 175 (57), 148 (38), 121 (40), 106 (100), 93 (26), 78 (66), 43 (74); MS CI *m/e* (relative intensity) 266 (M + 1, 4), 206 (50), 177 (100), 101 (16), 148 (45).

(G) 4-[(Acetoxymethyl)nitrosamino]-1-(3-pyridyl)-1-butanol (20). An excess of $NaBH_4$ (30 mg, 0.8 mmol) dissolved in 10 mL of 0.1 M Tris-HCl, pH 7, was added to 3 (0.22 g, 0.81 mmol) dissolved in 30 mL of 0.1 M Tris-HCl, pH 7.0, and stirred for 1 h. The solution was extracted with methylene chloride which was then dried over sodium sulfate, filtered, and evaporated to yield 0.15 g of 20 (0.56 mmol) in 70% yield: ^1H NMR ($CDCl_3$) 8.56 (2 H, m, 2,6-pyridyl), 7.71 (1 H, m, 4-pyridyl), 7.30 (1 H, m, 5-pyridyl), 6.15 [1.8 H, s, (*E*)-CH_2OAc], 5.32 [0.2 H, s, (*Z*)-CH_2OAc], 4.75 (1 H, m, methine), 3.61 (2 H, m, CH_2CH_2NNO), 2.11 [2.7 H, s, (*E*)-CH_3CO], 2.06 [0.3 H, s, (*Z*)-CH_3CO], 1.6 (4 H, m, $CHCH_2CH_2CH_2NNO$).

(H) 1-(3-Pyridyl)but-3-en-1-ol (25) and 1-(3-Pyridyl)but-2-en-1-ol (26). 2-(3-Pyridyl)-2-[(trimethylsilyl)oxy]-4-pentenonitrile (*12*) (100 mg, 0.4 mmol) was dissolved in 1 mL of chloroform and stirred vigorously with 1 mL of 0.5 N HCl at room temperature for 1 h. The pH of the mixture was brought to 1 by the addition of saturated sodium acetate solution. The aqueous layer was extracted with chloroform, and the combined organic extracts were dried over $MgSO_4$, filtered, and evaporated to give crude 1-(3-pyridyl)but-3-en-1-one. $NaBH_4$ (25 mg) was added to the crude product dissolved in 5 mL of 0.1 N Tris-HCl, pH 7, and the mixture was stirred for 2 h. The solution was extracted with chloroform, dried with $MgSO_4$, and evaporated. Compounds 25 and 26 were purified by silica gel HPLC on a 4.9 mm × 50 cm Whatman ODS-3 column with elution by 5% MeOH in methylene chloride at 4 mL/min in about 60% and 10% yields, respectively.

25: ^1H NMR ($CDCl_3$) δ 8.65 (1 H, br s, 6-pyridyl), 8.54 (1 H, br s, 2-pyridyl), 7.71 (1 H, d/t, 4-pyridyl), 7.29 (1 H, d/d, 5-pyridyl), 5.80 (1 H, m, 3-vinyl), 5.18 (1 H, m, 4-vinyl), 4.79 (1 H, d/d, 4-vinyl), 4.22 (1 H, m, methine), 2.50 (2 H, m, methylene); MS EI *m/e* (relative intensity) 149 (M^+, 42), 134 (100), 106 (78), 78 (40); MS CI 150 (M + 1, 100).

26: ^1H NMR ($CDCl_3$) δ 8.61 (1 H, br s, 6-pyridyl), 8.56 (1 H, br s, 2-pyridyl), 7.71 (1 H, d/t, 4-pyridyl), 7.29 (1 H, d/d, 5-pyridyl), 5.80 (1 H, m, 3-vinyl), 5.70 (1 H, m, 2-vinyl), 5.21 (1 H, d, methine), 1.75 (3 H, d, methyl); MS EI *m/e* (relative intensity) 149 (M^+, 50), 108 (100).

(I) 2-(3-Pyridyl)-2,3,4,5-tetrahydrofuran (24). *p*-Toluenesulfonyl chloride (0.24 g, 1.3 mmol) and 22 (0.19 g, 1.1 mmol) were dissolved in 2 mL of anhydrous pyridine at −20 °C and stirred overnight under a nitrogen atmosphere. The solution was then heated at 90 °C for 30 min to effect cyclization. The pyridine was removed in vacuo, and the residue was dissolved in methylene chloride, washed with H_2O, and then dried over $MgSO_4$. The product was purified by silica gel HPLC by elution with 5% MeOH in chloroform at 4 mL/min. It was obtained in about 50% yield: ^1H NMR ($CDCl_3$) δ 8.58 (1 H, br s, 6-pyridyl), 8.51 (1 H, d, 2-pyridyl), 7.64 (1 H, d/t, 4-pyridyl), 7.27 (1 H, d/d, 5-pyridyl), 4.90 (1 H, t, methine), 4.10 (1 H, d/d, 5-methylene), 3.94 (1 H, d/d, 5-methylene), 2.38 (1 H, m, 3-methylene), 2.02 (2 H, m,

4-methylene), 1.69 (1 H, m, 3-methylene); MS EI *m/e* (relative intensity) 149 (M^+, 80), 108 (100); MS CI 150 (M + 1).

(J) 1-(3-Pyridyl)-1,3-butanediol (27). An excess of $NaBH_4$ (40 mg, 1.2 mmol) was added to 19 (0.20 g, 1.2 mmol) in 10 mL of H_2O, and the mixture was stirred for 30 min at room temperature. The solution was extracted 15 times with ethyl acetate. The combined extracts were dried with $MgSO_4$, filtered, and evaporated to yield 27 in 75% yield: ^1H NMR ($CDCl_3$) δ 8.49 (1 H, m, 2-pyridyl), 8.40 (1 H, m, 6-pyridyl), 7.70 (1 H, m, 4-pyridyl), 7.25 (1 H, m, 5-pyridyl), 5.14 (0.6 H, d/d, 1-methine), 5.02 (0.4 H, d/d, 1-methine), 4.20 (0.4 H, m, 3-methine), 4.11 (0.6 H, m, 3-methine), 1.9 (2 H, m, methylene), 1.36 (0.6 H, d, methyl), 1.33 (0.4 H, d, methyl); MS EI *m/e* (relative intensity) 167 (M^+, 5), 149 (28), 122 (7), 108 (100), 80 (18); MS CI *m/e* (relative intensity) 168 (M^+ + 1, 100), 106 (60).

Solvolyses. (A) HPLC Conditions. The solvolysis reactions were analyzed by reverse-phase HPLC using a 3.9 mm × 25 cm μBondapak C18 column with elution by 20–70% MeOH in 20 mM potassium phosphate, pH 7.0, over 50 min at 1 mL/min. The eluant was monitored with a Waters Model 440 UV/visible detector operated at 254 nm. The chromatogram was recorded with a Hitachi integrator. The retention times (min) of the compounds are as follows: 22 (8.5), 27 (9.5), 7 (10.5), 23 (16), 10 (19), 8 (19), 25 (20), 18 (22), 6 (22.5), 26 (23), 3 (23), 12 (26), 24 (26), 4 (30), 17 (44), and 16 (45).

The products of the solvolyses were collected in order to determine their structures by NMR and MS. A semipreparative 4.9 mm × 50 cm Whatman ODS-3 reverse-phase HPLC was employed, using the above gradient at 4.0 mL/min.

(B) 4-[(Acetoxymethyl)nitrosamino]-1-(3-pyridyl)-1-butanone (3), 4-(Carbethoxynitrosamino)-1-(3-pyridyl)-1-butanone (4), 4-(3-Pyridyl)-4-oxobutyl *p*-Toluenesulfonate (16), and 4-[(Acetoxymethyl)nitrosamino]-1-(3-pyridyl)-1-butanol (20). A 10-μL aliquot of 50 mM 3, 4, 16, or 20 in *p*-dioxane was added to 1 mL of 10 mM MOPS and 150 mM NaCl, pH 7.4, with and without 4.9 M MeOH. In addition, 3 was solvolyzed in 9.8 M methanol and tosylate 16 was solvolyzed in buffer/methanol (9/1). The solvolysis of 16 was complete within 8 h. The solvolyses of 3 and 4 were initiated by the addition of an aliquot of esterase (60 units/mL). The hydrolysis of 3 was complete within 1 h of the addition of 30 μL of esterase. The hydrolysis of 4 was complete in 4 h by the addition of 100 μL of esterase. The solvolysis of 20 was begun by addition of 100 μL of esterase and was complete in 24 h.

The solvolysis reactions were monitored by reverse-phase HPLC as described above. The yields of the each product were determined by integration of the peak area. The identities of the products were determined by cochromatography with synthetic standards, as well as by MS and NMR analysis of isolated peaks.

Solvolyses of 3, 4, and 20 were also performed in deuterated buffer. The buffer was prepared by dissolving the MOPS (for 3, 0.01, 0.1, or 1 M; for 4 and 20, 0.01 M) and NaCl in D_2O and then adjusting the pH to 7.0 with NaOD. This reading on the pH meter is equivalent to a pD of 7.4 (20). The products were isolated by the HPLC method described above and analyzed for deuterium incorporation by NMR and MS.

(C) 2-Chloro-2-(3-pyridyl)-2,3,4,5-tetrahydrofuran (17). A 10-μL aliquot of 50 mM 17 in *p*-dioxane was added to either H_2O or 4.9 M MeOH in H_2O. The solvolysis was begun by the addition of 10 μL of 100 mM $AgNO_3$. The reaction products were monitored as described above for the solvolyses of compounds 3, 4, 16, and 20.

Results

The model compounds for NNK were prepared as described under Experimental Section. Standards for identification of potential solvolysis products were prepared independently in order to compare their NMR, MS, and HPLC retention times with those of the products of the solvolyses reactions. The synthesis of 8 was unexpectedly difficult. The attempted methylation of the keto alcohol 7 with methyl iodide or methyl triflate in anhydrous THF or benzene using triethylamine, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), or sodium hydride as a base resulted in the formation of 4-hydroxy-2-methyl-1-(3-pyridyl)-1-butanone. The reaction of the tosylate 16 in MeOH with $NaHCO_3$, Na_2CO_3, or triethylamine resulted in the formation of the cyclopropyl ketone 10. The methyl ether 8 was finally synthesized by reacting 1-iodo-3-methoxypropane with the anion of 2-(3-pyridyl)-2-[(trimethylsilyl)oxy]acetonitrile. The resulting 5-methoxy-2-(3-pyridyl)-2-[(trimethylsilyl)oxy]pentanonitrile was deprotected with HCl to form 8. The reduction of 1-(3-pyridyl)but-3-en-1-one at pH 7 produced a mixture of 1-(3-pyridyl)but-2-en-1-ol (26) and 1-(3-pyridyl)but-3-en-1-ol (25). The compounds were purified by HPLC, and the ^1H NMR spectra provided unambiguous characterization of each isomer. The doublet methyl group peak and the integration of two vinyl proton signals of 26 contrasted to the three vinyl proton signals and complex methylene peaks of 25.

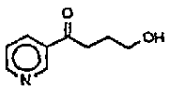
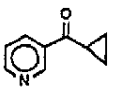
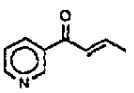
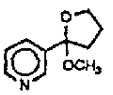
The results of the solvolyses of the pyridyloxobutylating agents are presented in Table I and illustrated in Scheme II. In all cases the keto alcohol 7 was the major product. The ketocyclopropyl derivative 10 and the α,β-unsaturated ketone 18 were also observed in the hydrolysis mixture from the α-acetoxy nitrosamine 3 and carbamate 4. Only compound 4 decomposed to myosmine 6 in 28 and 38% yields in aqueous and methanolic buffers, respectively. When the α-acetoxy nitrosamine 3, carbamate 4, tosylate 16, or cyclic chloride 17 was solvolyzed in the presence of MeOH, the cyclic methyl ketal 12 was formed, but 4-methoxy-1-(3-pyridyl)-1-butanone (8) was not detected as a product.

Since methyl ether 8 and cyclopropyl ketone 10 coeluted in the HPLC system, the determination of the yield of 8 was complicated. The identity of the peak was assigned as compound 10, because the size of the peak was independent of the amount of MeOH present and the EI and CI MS matched that of 10 and not 8. The detection limit of a 0.0005% yield was based on the CI MS. In an attempt to increase the formation of the methyl ether 8, nitrosamine 3 was solvolyzed in 40% methanol (higher concentrations caused denaturation of the esterase) and the tosylate 16 was solvolyzed in 90% methanol. The yield of the cyclic ketal 12 increased to 20% in the hydrolysis of 3 and 85% in the hydrolysis or 16. No methyl ether was detected. We therefore conclude that little if any methyl ether 8 was formed in the hydrolysis of these compounds.

The solvolysis of the α-acetoxy derivative of NNAL (20) was studied to investigate the possible cyclization pathway in the metabolism of NNK and NNAL. The results of the solvolyses in the absence and presence of MeOH are presented in Table II and illustrated in Scheme III. The major products were the tetrahydrofuran derivative 24 resulting from intramolecular cyclization and the 1,4-diol 22. The presence of MeOH did not affect the product distribution to a great extent, as the methyl ether 23 was formed in only 1% yield. The secondary ether 3-methoxy-1-(3-pyridyl)-1-butanol was not detected as a product.

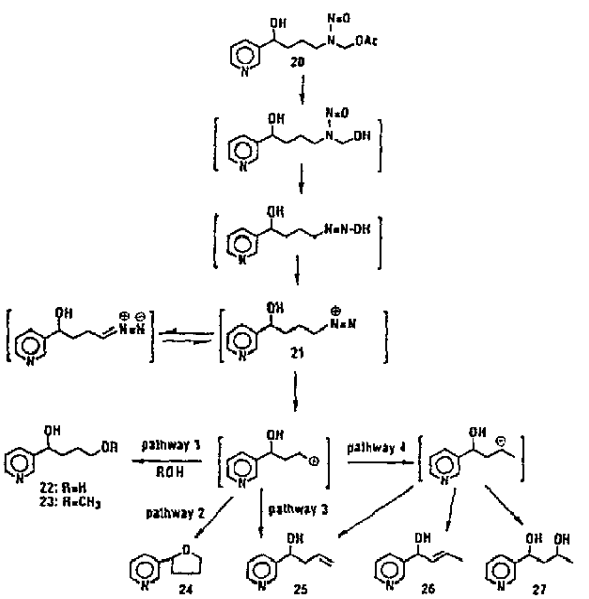
The nitroso compounds 3, 4, and 20 were solvolyzed in deuterated buffer in order to investigate the possible intermediacy of a diazonium ion. The proton on the carbon adjacent to the diazonium ion has a pK_a around 10 (21), and if the diazonium ion has an appreciable lifetime, those hydrogen atoms will exchange with the solvent (22). Keto alcohol 7 was isolated from the hydrolysis of compound 3 in three different concentrations of MOPS in 150 mM NaCl, pD 7.4, in D_2O. The total deuterium incorporation into the keto alcohol 7 was as follows: 0.010 M, 3.9%; 0.10 M, 6%; 1.0 M, 27%. The deuterium incorporations in the products of the hydrolysis of the α-acetoxy NNAL deriv-

Table I. Solvolysis of Model Pyridyloxobutylating Agents[a]

products		3[b,c]		4[b,d]		17[b]		18[e]	
		H_2O	MeOH[f]	H_2O	MeOH[f]	H_2O	MeOH[f]	H_2O	MeOH[f]
7		63	48	48	28	100	78	100	80
10		4	4	2	3	0	0	0	0
18		24	30	17	13	0	0	0	0
12			11		13		22		20

[a] Product distribution expressed in percent yield. Concentration of substrate is 0.5 mM. [b] Solvolyzed in 10 mM MOPS and 150 mM NaCl, pH 7.4 at 22 °C. [c] Contained 5.7 μg/mL esterase. [d] Contained 19 μg/mL esterase. [e] Solvolyzed in 1 mM $AgNO_3$. [f] Contained 4.9 M methanol.

Scheme III

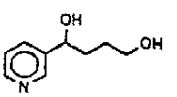
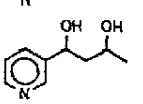
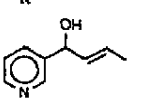
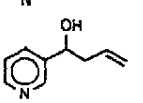
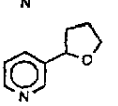
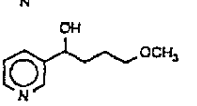


ative **20** in 10 mM MOPS and 150 mM NaCl, pD 7.4, in D_2O at 22 °C as determined by CI MS were as follows: **22**, 2.1%; **27**, 2.6%; **26**, 0.8%; **24**, 3.0%.

Discussion

α-Hydroxylation of the methyl carbon of NNK is expected to lead to diazonium ion **2**, which can react with cellular nucleophiles. This species alkyates DNA to form an unidentified unstable adduct (**5**). In order to gain an understanding of the nature of this adduct, the analysis of the products of the solvolysis of model compounds for **2** was undertaken. The model compound **3** generates the *initial hydroxylated* metabolite 4-[(hydroxymethyl)-nitrosamino]-1-(3-pyridyl)-1-butanone (**1**) upon esterase hydrolysis. This product is expected to decompose to 4-oxo-4-(3-pyridyl)-1-butanediazohydroxide (**5**) and formaldehyde. The diazohydroxide **5** can also be formed upon esterase hydrolysis of the carbamate **4**. Consistent with the formation of a common intermediate in the hydrolysis of **3** and **4** is their similar spectrum of products (Scheme II).

Table II. Solvolysis of 4-[(Acetoxymethyl)nitrosamino]-1-(3-pyridyl)-1-butanol (20)[a]

product		H_2O	MeOH[b]
22		47	43
27		7	3
26		10	12
25		5	6
24		31	36
23			1

[a] Solvolyzed in 0.50 mM **20**, 10 mM MOPS, and 150 mM NaCl, pH 7.4 at 22 °C, containing 19 μg/mL esterase. Product distribution expressed in percent yield. [b] Contained 4.9 M MeOH.

The keto alcohol 7 was the major hydrolysis product of the α-acetoxy nitrosamine 3 and carbamate 4. This observation was previously reported for compound 4 (*10*, *13*). We also observed the formation of the α,β-unsaturated ketone 18 and the cyclopropyl ketone 10. Mysomine (6) was only detected in the mixture from 4, indicating that the alkanediazohydroxide 5 is not a precursor to 6 as previously suggested (*10*).

Evidence for the formation of the diazonium ion was found in the hydrolyses of the nitrosamines 3 and 4 in deuterated buffer (*21*, *22*). The amount of deuterium incorporation was proportional to the concentration of buffer, indicating that the exchange of these protons was general base catalyzed. These results suggest that both 3 and 4 hydrolyze via the diazonium ion intermediate 2.

The formation of keto alcohol 7, cyclopropyl ketone 10, and olefin 18 suggests that the diazonium ion 2 can decompose via several pathways (*23*) (Scheme II). First, the alkanediazonium ion (or primary carbonium ion) can react directly with the solvent to yield keto alcohol 7. Second, the reactive species could cyclize by intramolecular attack of the carbonyl oxygen on the electrophilic terminal carbon atom to form the cyclic oxonium ion 13. This species could react with H_2O to generate the unstable hemiacetal 11 which would subsequently rearrange to 7. Third, the reactive species could eliminate nitrogen and a proton to generate a terminal olefin. However, the terminal olefin 14 would be expected to rearrange to the α,β-unsaturated ketone 18. Fourth, the diazonium ion (or primary carbonium ion) could also rearrange to form the secondary carbonium ion 15. 3-Hydroxy-1-(3-pyridyl)-1-butanone (19) was not observed as a product; however, it is likely that a proton is lost, to yield the α,β-unsaturated ketone 18. Fifth, deprotonation of the carbon atom adjacent to the carbonyl group can produce a species that undergoes intramolecular reaction, with the electrophilic 4-carbon atom forming the cyclopropyl ketone 10.

Since the major decomposition product of the nitrosamines 3 and 4 is the keto alcohol 7, the diazonium ion 2 (or primary carbonium ion) must decompose primarily via pathway 1 or 2. The importance of the cyclic oxonium ion 13 in the hydrolysis reactions was demonstrated when the solvolysis of 3 or 4 was conducted in the presence of 20% MeOH. The oxonium ion was trapped by MeOH, resulting in the formation of the stable acetal 12. No 4-methoxy-1-(3-pyridyl)-1-butanone (8) was detected from the methanolysis of the primary carbonium ion 9. These results suggest that the major hydrolysis pathway of the alkanediazonium ion 2 proceeds through the cyclic oxonium ion 13 (pathway 2).

The intermediacy of the cyclic oxonium ion was further supported by the results obtained in the solvolyses of the keto tosylate 16 and cyclic chloride 17. These derivatives are models for species further along the reaction pathway than the nitroso compounds 3 or 4. Keto tosylate 16 is a model for the diazonium ion 2; however, the tosyl moiety is a poorer leaving group than molecular nitrogen. Thus, the hydrolysis of 16 is not as likely to involve a carbonium ion intermediate. The cyclic chloride 17 is a direct precursor to the cycic oxonium ion and was not expected to hydrolyze via an acyclic pathway. The solvolyses of the two compounds gave essentially identical yields of products, implicating a common intermediate in the solvolyses. These results demonstrate that the 1-oxo-1-(3-pyridyl)-butyl group with a leaving group in the 4-position readily cyclizes to form the cyclic oxonium ion 13.

Both reaction pathways 1 and 2 are important in the hydrolysis of the alkanediazonium ion generated from the α-acetoxy derivative of NNAL, 20 (Scheme III). Decomposition of the diazonium ion 21 via pathway 1 gives the major product, the 1,4-diol 22. Intramolecular cyclization of the diazonium ion (pathway 2) generates the tetrahydrofuran derivative 24, which is also a prominent product. The reasons why 20 proceeds through pathway 1 to a greater extent than do compounds 3, 4, 16, and 17 are unclear.

When compound 20 was hydrolyzed in the presence of 20% MeOH, 4-methoxy-1-(3-pyridyl)-1-butanol (23) was observed. However, the yield of this solvolysis product (1%) was unexpectedly low. This observation contrasts with those from the solvolyses of 3 and 4 in which the oxonium ion reacts extensively with both H_2O and MeOH. The low extent of reaction with MeOH is consistent with the formation of a tight ion pair between the diazonium ion 21 and hydroxide ion, generating the 1,4-diol 22, before the solvent can react with the diazonium ion (*24*, *25*). These observations provide evidence that methyl hydroxylation of NNAL leads to a diazonium ion capable of reacting with nucleophiles, albeit in low yields. It is possible that α-hydroxylation of NNAL produces DNA pyridylhydroxybutylation.

Minor products were also observed in the hydrolysis reactions of 20. The formation of 25 suggests the involvement of a direct elimination pathway (pathway 3). The ability of diazonium ions to undergo elimination reactions has been suggested by previous studies (*23*, *26*). Rearrangement to a secondary carbocation (pathway 4) also occurs; evidence for this is formation of the internal olefin 26 and the 1,3-diol 27. The terminal olefin 25 could also be formed via this pathway. No cyclopropyl derivative was observed in the reactions of 20, suggesting that the carbonyl function of the diazonium ion 2 activates the neighboring carbon for nucleophilic attack on the terminal carbon atom to form the cyclopropyl ketone 10.

These solvolysis studies have demonstrated that methyl hydroxylation of NNK or NNAL leads to the formation of a variety of electrophilic species. In the case of NNK, α-hydroxylation of the methyl group leads to the production of the diazonium ion 2, which then undergoes intramolecular displacement of molecular nitrogen by the carbonyl oxygen to form a reactive cyclic oxonium ion, 13. Our solvolysis studies suggest that H_2O and MeOH primarily react with the cyclic oxonium ion 13, and not the keto diazonium ion 2. However, MeOH and H_2O are relatively weak nucleophiles. When the alkylation of *N*-acetyl-L-cysteine, a powerful nucleophile, by 3, 4, 4-oxo-4-(3-pyridyl)-1-butyl methanesulfonate, and 4-iodo-1-(3-pyridyl)-1-butanone was investigated by Carmella and co-workers (*11*), they observed the formation of both the straight-chain adduct *S*-[4-oxo-4-(3-pyridyl)-1-butyl]-*N*-acetyl-L-cysteine and the cyclic adducts (*R*)- and (*S*)-*S*-[2-(3-pyridyl)-2,3,4,5-tetrahydrofuran-2-yl]-*N*-acetyl-L-cysteine. These results are consistent with the formation of diazonium ion 2 which can react directly with strong nucleophiles or cyclize to the cyclic oxonium ion 13 which reacts to form labile ketal derivatives. Therefore, the nature of the alkylation product depends heavily on the nucleophilicity of the target.

DNA contains a variety of sites with differing degrees of reactivity. The 7-position of guanine could react to form both straight-chain and cyclic pyridyloxobutyl adducts while weaker nucleophiles such as the O^6- or N^2-position of guanine might form primarily cyclic adducts. The potentially labile nature of such cyclic adducts might explain the difficulties encountered in the isolation of the pyridyloxobutyl adduct(s) of NNK. It is possible that the

presence of keto alcohol 7 in the hydrolysates of DNA isolated from rats treated with [5′-^{3}H]NNK (5) results from hydrolysis of an unstable cyclic adduct. Experiments are underway to determine whether DNA reacts with the cyclic oxonium ion in vivo to produce labile ketal derivatives.

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